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Effect of local application of the antimicrobial peptide IB-367 on the incidence and severity of oral mucositis in hamsters.

Loury D, Embree JR, Steinberg DA, Sonis ST, Fiddes JC.

IntraBiotics Pharmaceuticals, Inc., Mountain View, Calif 94043-1833, USA.

OBJECTIVE: The purpose of this animal study was to determine whether IB-367, an antimicrobial peptide, is able to ameliorate oral mucositis by reducing microflora densities on the mucosal surfaces of the mouth. STUDY DESIGN: Oral mucositis was induced in hamsters by intraperitoneal injection of 5-fluorouracil followed by superficial abrasion of the buccal mucosa. A test formulation was applied topically to the buccal mucosa 5 or 6 times per day starting 6 to 8 hours before abrasion. RESULTS: Mucositis scores were significantly lower ($P < .05$) in hamsters given formulations containing 0.5 or 2.0 mg/mL of IB-367 than in placebo-treated controls. Treatment with IB-367 produced a more than 100-fold reduction in oral microflora densities. In a second experiment, treatment of hamsters with a formulation containing IB-367 at 0.12, 0.5 or 2.0 mg/mL resulted in a dose-dependent reduction in mucositis severity. CONCLUSION: The results indicate that reduction of local microflora densities through use of IB-367 may improve clinical outcomes in patients at risk for the development of oral mucositis.

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Prognostic assessment of 2,361 patients who underwent pulmonary resection for non-small cell lung cancer, stage I, II, and IIIA.

van Rens MT, de la Riviere AB, Elbers HR, van Den Bosch JM.

Departments of Pulmonary Diseases, Sint Antonius Hospital, Nieuwegein, The Netherlands.

STUDY OBJECTIVES: Staging and classification in lung cancer are important for both patient management and clinical research. Results of survival after resection in patients with primary non-small cell lung cancer (NSCLC) are analyzed in order to validate recent refinements of the staging system. DESIGN: Retrospective study; period from 1970 to 1992; follow-up > or = 5 years. PATIENTS: A total of 2,361 previously untreated patients who underwent resection for stage I, II, or IIIA primary NSCLC. MEASUREMENTS: Survival was estimated from the date of operation using the Kaplan-Meier survival analysis method. Deaths within 30 days of operation were excluded. Survival comparisons of different surgical-pathologic TNM classification (based on pathologic examination of resected specimens) as well as further discriminative factors were analyzed by log-rank test. RESULTS: Postoperative death occurred in 3.9% of patients. For survival analyses, 2,263 patients were included. The overall 5-year survival was 937/2,263 (41.4%). Five-year survival in stage IA was 255/404 (63%); in stage IB, 367/797 (46%); in stage IIA, 43/83 (52%); in stage IIB, 210/642 (33%); and in stage IIIA, 63/337 (19%). No significant difference in survival was demonstrated between stages IB and IIA. Until 4 years after surgery, age at operation did not influence survival; after 5 years, patients > 65 years old had a significantly lower survival. CONCLUSION: The TNM staging system accurately reflects the prognosis in primary NSCLC, but some stage definitions can be discussed. Despite the fact that the staging system is built on clinical data, the present analysis, which includes postsurgical data, confirms the similar survival of patients with T2N0M0 and T1N1M0. These results also stress the use of two separate substages, especially because these patients are offered surgery when

Prognostic Assessment of 2,361 Patients Who Underwent Pulmonary Resection for Non-small Cell Lung Cancer, Stage I, II, and IIIA*

Marcel Th. M. van Rens, MD; Aart Brutel de la Rivière, MD, PhD, FCCP; Hans R. J. Elbers, MD, PhD; and Jules M. M. van den Bosch, MD, PhD, FCCP

Study objectives: **Staging and classification in lung cancer are important for both patient management and clinical research. Results of survival after resection in patients with primary non-small cell lung cancer (NSCLC) are analyzed in order to validate recent refinements of the staging system.**

Design: **Retrospective study; period from 1970 to 1992; follow-up ≥ 5 years.**

Patients: **A total of 2,361 previously untreated patients who underwent resection for stage I, II, or IIIA primary NSCLC.**

Measurements: **Survival was estimated from the date of operation using the Kaplan-Meier survival analysis method. Deaths within 30 days of operation were excluded. Survival comparisons of different surgical-pathologic TNM classification (based on pathologic examination of resected specimens) as well as further discriminative factors were analyzed by log-rank test.**

Results: **Postoperative death occurred in 3.9% of patients. For survival analyses, 2,263 patients were included. The overall 5-year survival was 937/2,263 (41.4%). Five-year survival in stage IA was 255/404 (63%); in stage IB, 367/797 (46%); in stage IIA, 43/83 (52%); in stage IIB, 210/642 (33%); and in stage IIIA, 63/337 (19%). No significant difference in survival was demonstrated between stages IB and IIA. Until 4 years after surgery, age at operation did not influence survival; after 5 years, patients > 65 years old had a significantly lower survival.**

Conclusion: **The TNM staging system accurately reflects the prognosis in primary NSCLC, but some stage definitions can be discussed. Despite the fact that the staging system is built on clinical data, the present analysis, which includes postsurgical data, confirms the similar survival of patients with T2N0M0 and T1N1M0. These results also stress the use of two separate substages, especially because these patients are offered surgery when possible.**

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Key words: lung neoplasm; neoplasm staging; non-small cell lung carcinoma; prognosis; pulmonary surgical procedures

Abbreviations: NSCLC = non-small cell lung carcinoma; pTNM = surgical-pathologic TNM classification (estimate of disease extent based on pathologic examination of resected specimens)

Staging and classification in lung cancer are important for both patient management and clinical research.[1–3] Recently, the staging system for lung cancer was updated and refined based on clinical data.[2] Patients with non-small cell lung carcinoma (NSCLC) and limited disease are offered surgery when possible, and postsurgical data also support the revised staging system.[2]

Our own data from patients who underwent surgery are comparable in numbers as well as in follow-up to those recently presented,[2] so we analyzed them for validation. Adherence to the new system is important because many new treatment modalities have been proposed recently. These new strategies may improve survival, but this can only be determined properly if the new staging system is generally accepted and based on large numbers of patients.

We present in this study our results of survival after lung resection in patients with primary non-

*From the Departments of Pulmonary Diseases (Drs. van Rens and van den Bosch), Thoracic Surgery (Dr. Brutel de la Rivière), and Pathology (Dr. Elbers), Sint Antonius Hospital, Nieuwegein, The Netherlands.

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Correspondence to: Jules M.M. van den Bosch, MD, PhD, FCCP, Sint Antonius Hospital, Department of Pulmonary Diseases, PO Box 2500, 3430 EM Nieuwegein, The Netherlands; e-mail: antolong@knmg.nl

small cell lung cancer in stages I, II, and IIIA, in order to discuss the recent refinements of the staging system.[2]

Materials and Methods

From 1970 through 1992, 2,799 patients underwent surgery for NSCLC, 2,559 of whom underwent resection. Of this latter group, 2,361 patients underwent resection for stage I, II, or IIIA primary NSCLC.[1] None of the patients had received previous treatment for NSCLC. Staging definitions for the T (primary tumor), N (regional lymph nodes), and M (distant metastasis) components were used according to the International Staging System for Lung Cancer.[1]

Patient age ranged from 26 to 85 years, with a median of 64.2 years. Patients were > 65 years old at the time of surgery in 1,115 cases (47.2%). There were 2,196 men (93%) and 165 women. The number of women who underwent resection for stage I, II, and IIIA NSCLC increased from 3.9% in 1970 to 1974 to 9.4% in 1990 to 1992. Surgical-pathologic TNM classification (pTNM) of the studied patients is given in Table 1.

Tumors were located in the right lung in 1,266 patients (53.6%) and in the left lung in 1,095. Histologic typing was done according to the World Health Organization classification.[4] Tumors were histologically classified as squamous cell carcinoma in 1,607 patients (68.1%), adenocarcinoma in 542 (23.0%), adenosquamous in 88 (3.7%), and undifferentiated large cell carcinoma in 124 (5.2%) patients. Cervical mediastinoscopy was performed in 2,263 patients (95.8%). In 37 patients, a left parasternal mediastinotomy was performed. A pneumonectomy was performed in 610 patients; lobectomy, 1,390 patients; bilobectomy, 203 patients; segmental resection, 142 patients; and wedge resection, 16 patients. Additional lung resection (segmental or wedge) was performed in 57 patients. Bronchoplastic surgery was performed in 179 patients. Sixty-four patients underwent concurrent surgery for heart disease. Resection was considered complete in 89.9% of the patients because (1) the surgeon was morally certain that all known disease was removed; (2) resection margins were free of disease on pathologic examination; and (3) the highest mediastinal lymph node was negative by microscopy.[5] Completeness of surgery listed by pTNM status is given in Table 1.

Survival was estimated from the date of operation using the Kaplan-Meier survival analysis method.[6] Deaths within 30 days of operation were excluded. Survival comparisons of different pTNM classifications as well as further discriminative factors were analyzed by log-rank test.[7] The difference was considered significant when the p value was < 0.05.

Table 1—*pTNM Classification of Patients (n = 2,361) and Completeness of Resection**

pTNM	No. of Patients	% of Total	Complete Resection (%)
T1N0M0	416	17.6	98.8
T2N0M0	833	35.3	95.2
T1N1M0	84	3.6	100.0
T2N1M0	541	22.9	94.1
T3N0M0	137	5.8	67.2
T3N1M0	89	3.8	71.9
T1N2M0	13	0.6	100.0
T2N2M0	187	7.9	69.0
T3N2M0	61	2.6	45.9

*According to Pitz et al.[5]

Results

Within 30 days after surgery, 91 patients died (3.9%), 43 of whom patients had undergone pneumonectomy. Postoperative death was related not to disease stage but to the extension of the resection (7.2% for pneumonectomy; 5.4% for bilobectomy; 2.5% for lobectomy; 0.6% for small resections) and the patient's age (6.4% in elderly patients and 2.1% in patients aged < 65 years). Seven patients were lost to follow-up; therefore, 2,263 patients were included for survival analyses. Overall 5-year survival was 937/2,263 (41.4%).

The best 5-year survival rate (63%) was seen in pT1N0M0, and the worst survival (7%) in pT3N2M0. Survival rates listed by the pTNM status are presented in Table 2. There were significant differences ($p < 0.0001$) in survival between pT1N0M0 (n = 404) and pT2N0M0 (n = 797). Survival after resection was better in pT1N1M0 (n = 83) than in pT2N0M0 (n = 797); however, the difference was not significant ($p = 0.73$). Patients with pT3N0M0 disease (n = 132) showed significantly better survival ($p < 0.002$) than patients with pT3N2M0 (n = 57) and pT2N2M0 (n = 180) disease, but the difference in survival between pT3N0M0 and pT3N1M0 (n = 87) was not significant ($p = 0.31$). Using the 1997 staging criteria,[2] significant differences in survival were demonstrated between stages IA and IB, between stages IIA and IIB, and between stages IIB and IIIA. No significant difference was demonstrated between stages IB and IIA. Five-year survival rates are shown in Table 3 and Figure 1. Long-term survival for different stages is given separately in Table 4.

Various factors possibly influencing postoperative survival were investigated. Survival in patients with complete resection was significantly better ($p < 0.0001$) than survival in patients with incomplete resection, which was demonstrated more frequently in advanced disease. Five-year survival was 44.3% in patients with complete resection vs 16.2% in patients with incomplete resection. Significant differences for this variable were demonstrated in pT2N0M0, pT2N1M0, pT3N0M0, and pT2N2M0. All patients with pT1N1M0 and pT1N2M0 were considered to have been treated with complete resection.

Survival was significantly better in patients who had squamous cell lung carcinoma compared with patients who had nonsquamous cell carcinoma in the pT2N1M0 subset ($p < 0.0005$); this difference was not found in other pTNM subsets.

As shown in Figure 2, patients aged < 65 years (median age, 58.5 years; n = 1,246) at operation had significantly better survival compared with patients

Table 2—*Survival Rates (Cumulative Percentage Surviving)**

pTNM	Study*	No. of Patients (No. Evaluable)†	Time After Treatment, mo				
			12	24	36	48	60
T1N0M0	**VR**	**416 (404)**	**94**	**83**	**76**	**69**	**63**
	M	511	94	86	80	73	67
T2N0M0	**VR**	**833 (797)**	**85**	**68**	**59**	**52**	**46**
	M	549	87	76	67	62	57
T1N1M0	**VR**	**84 (83)**	**90**	**72**	**61**	**57**	**52**
	M	76	89	70	64	61	55
T2N1M0	**VR**	**541 (510)**	**76**	**56**	**44**	**38**	**33**
	M	288	78	56	47	42	39
T3N0M0	**VR**	**137 (132)**	**64**	**47**	**40**	**34**	**33**
	M	87	76	55	47	40	38
T3N1M0	**VR**	**89 (87)**	**68**	**38**	**32**	**28**	**25**
	M	55	65	38	30	30	25
T1N2M0	**VR**	**13 (13)**	**92**	**77**	**69**	**38**	**31**
	M	53‡	84	64	52	45	38
T2N2M0	**VR**	**187 (180)**	**64**	**37**	**29**	**22**	**18**
	M	237‡	60	38	31	24	22
T3N2M0	**VR**	**61 (57)**	**49**	**21**	**12**	**12**	**7**
	M	56‡	59	26	16	12	12

*VR = data of present study, shown in bold; M = data published by Mountain.[2]
†Number of evaluable patients not available for study by Mountain.[2]
‡Data provided in personal communication (January 1998) with C.F. Mountain.

aged > 65 years (median age, 70.0 years; n = 1,017). The overall 5-year survival of patients < 65 years was 44%, compared with 38% for older patients ($p < 0.0001$). Taking the TNM subsets into account, this was demonstrated in the pT1–3N0M0 subsets ($p < 0.005$) and the pT2N1M0 subset ($p < 0.05$).

Discussion

Lung cancer staging—based on anatomic extent of the disease and using the TNM classification system—is an important aid to determine the clinical course of the patient and the success of treatment.[1–3,8] For patients with NSCLC, surgery and complete removal of the primary tumor and its involved lymph nodes remains the most effective mode of treatment.[9] The postresection survival of our patients with primary NSCLC of stages I, II, and IIIA is comparable to the survival reported by other investigators.[2,8,10] Apart from survival, we have compared the reported patient groups, as well as the methods used.

Compared with the surgical data published by Mountain,[2] the overall survival in the patients in our study was lower, albeit not significantly different (χ^2 test, results not shown). This minor difference may be caused by the pTNM subset distribution: In our study, the number of patients with advanced lung cancer is slightly larger. Inclusion of a relatively large number of patients with limited disease may result in increased overall survival rates. In analysis of specific pTNM subsets, survival in our patients is still slightly inferior to the results described by Mountain,[2] although again the differences were not significant. Inoue et al[8] reported better survival rates in patients who underwent curative operation than the survival results published by Mountain[2] and in the present

Table 3—*Survival Rates (Cumulative Percentage Surviving) in Different Stages**

Stage	No. of Patients (No. Evaluable)	Time After Treatment, mo					p Value (Log-Rank)
		12	24	36	48	60	
IA	416 (404)	94	83	76	69	63	
IB	833 (797)	85	68	59	52	46	<0.0001, IA vs IB
IIA	84 (83)	90	72	61	57	52	<0.02, IA vs IIA; 0.73 (not significant), IB vs IIA
IIB	678 (642)	74	54	43	37	33	<0.02, IIA vs IIB
IIIA	350 (337)	63	36	28	22	19	<0.0001, IIB vs IIIA

*Stages based on Mountain.[2]

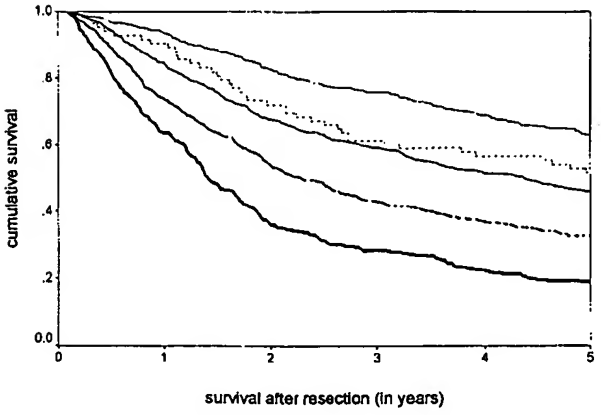

FIGURE 1. Survival curves for different stages according to Mountain.[2] Survival curves defined, from top to bottom: thin solid line = stage IA; thin dotted line = stage IIA; intermediate solid line = stage IB; thick dotted line = stage IIB; thick solid line = stage IIIA.

study. Detailed comparative analysis of the data of Inoue et al[8] was not possible because 5-year survival rates were reported in only a few pTNM subsets.[8] However, Mountain did not state the definition of "complete resection"; the stage I and IIA patients he reported on had all undergone complete resection. If we exclude the patients with incompletely resected tumors (n = 44) in these stages from our survival analysis, the 5-year survival rates do not change much (5-year survival in stage IA, 63%; stage IB, 47%; stage IIA, 52%) and are still poorer than those reported by Mountain.[2]

Studies investigating survival in NSCLC have reported that survival is likely to be determined by many factors, including the diversity of patients within one TNM subset, patient characteristics (*eg*, age), policy-changing preoperative investigations (*eg*, mediastinoscopy), indication for surgery, treatment after surgery, inclusion of multiple lung carcinoma, and probably histology.[5,11–17] In order to search for groups of patients who may benefit from surgery, different factors were analyzed.

The merits of the TNM classification to predict prognosis have been proved.[2,3,9] Although definitions of the T and N parameters are strict, each TNM subset will still contain a variety of patients because of differences in the exact anatomic spread of the tumor and in surgical radicality.[5,13,15,18,19] Therefore, a wide range of survival rates can be found within a TNM subset.

Surgery in older patients has been a subject of concern and may be related to postoperative mortality or poor survival outcome, especially in advanced cancer.[11,20] In the present study, elderly patients (≥ 65 years old) had a shorter survival. Decreased life expectancy contributes to the poorer survival, because the terminal event is defined as death by any cause. In this study, survival rates are very similar in older and younger patients until 4 years after surgery (44% vs 48%; Fig 2). After this period, there is an increased mortality in the elderly, probably because of increased age and comorbidity. This is supported by the finding that the difference in survival is only found in those pTNM subsets that have a better prognosis (*ie*, elderly patients can develop other diseases, resulting in death, although their surgery for lung cancer had been curative). This observation corroborates a more aggressive approach in the older patient, despite increased postoperative mortality.

Survival in relation to histology of NSCLC has been shown previously. Al-Kattan et al[11] reported no significant difference in 5-year survival for squamous cell carcinoma and adenocarcinoma in stages I to IIIA. We have found better survival in squamous cell carcinoma only for pT2N1M0. In the past, part of this material was investigated.[19] In this cohort of patients, reanalysis showed no relationship between nodal involvement and histology or tumor size (results not shown).

Mediastinoscopy was included in the preoperative assessment of our patients with NSCLC. Patients with positive mediastinoscopy were generally excluded from surgery; therefore, we included in our series a relatively small number of patients with N2 disease (261/2,361; 11%). In the study by Mountain,[2] mediastinoscopy was not performed on a routine

Table 4—*Long-term Survival Rates (Cumulative Percentage Surviving) in Different Stages**

Stage	No. of Patients (No. Evaluable)	Time After Treatment, yr†				
		5	10	15	20	25
IA	416 (404)	63 [255]	38 [136]	22 [66]	12 [16]	6 [5]
IB	833 (797)	46 [367]	26 [171]	15 [72]	9 [28]	7 [6]
IIA	84 (83)	52 [43]	28 [16]	17 [6]	5 [1]	—
IIB	678 (642)	33 [210]	19 [86]	11 [33]	6 [9]	5 [4]
IIIA	350 (337)	19 [63]	11 [24]	3 [4]	1 [1]	1 [1]

*Stages per Mountain.[2]

†Number in brackets represents the number of patients alive at defined moment (survival analysis after 5 years included censored cases).

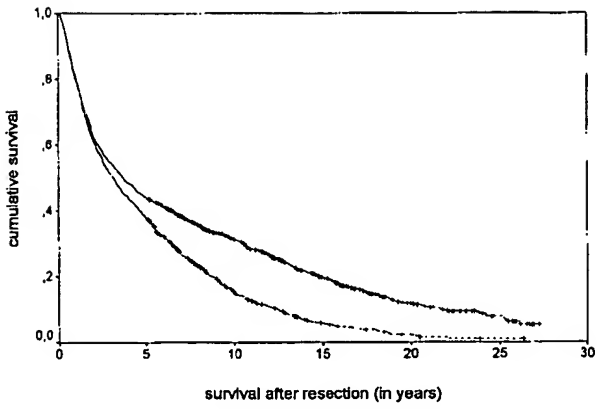

FIGURE 2. Survival curves of overall survival of patients aged < 65 years (mean survival, 6.34 years) and > 65 years (mean survival, 4.61 years). Solid line = patients aged < 65 years at operation; dotted line = patients aged > 65 years; + = censored case.

basis, and more patients with N2 disease were included (346/1,912; 18%). Although survival generally is found to be better in patients with intraoperatively diagnosed N2 disease, in our study population, survival in N2 disease was lower, but not significantly different.[2,14,21,22]

Also, the impact of postoperative therapy—which was rather diverse and changed over time in our patients—may have resulted in a different overall survival. Generally, patients with incomplete resection were treated with postoperative radiotherapy. Patients with N2 disease, as well as a considerable number of patients with NSCLC and chest wall involvement, were also treated with radiotherapy postoperatively. The benefit of radiotherapy in the individual patient is difficult to assess.[23] Pitz et al[5] analyzed 125 patients with NSCLC and chest wall involvement who underwent resection. Postoperative radiotherapy had no effect on survival in these patients. Changes in use of (new) chemotherapeutics and alterations of radiotherapeutic regimes may change survival of patients with NSCLC and are of interest in patients with either limited or advanced disease.

The indication for surgery, and whether the procedure is radical or not, also have an impact on the survival rates. In the last decade, more aggressive surgery and more liberal inclusion of patients with advanced disease have been noted. An individual patient may benefit, but in general this policy will result in a lower overall survival rate in these subsets. This is supported by the survival rates published by Mountain in 1997,[2] which are slightly lower than those published in 1986.[1] Most prominent is the decline in survival of patients with N2 disease: in 1986, Mountain reported a 5-year survival of 28.8% in this group,[1] but in 1997, he reported a 23% 5-year survival.[2] Patients with N2 disease are frequently offered surgery today. More aggressive neoadjuvant therapeutic regimens are likely to improve survival rates after surgery by downstaging the disease after chemotherapy.

Patients with synchronous multiple lung carcinoma have a worse prognosis, and the definition of multiple lung carcinoma is still under discussion. These patients were not included in this study. In the new staging system, such tumors may be classified as stage IIIB or stage IV disease.[2]

Concerning the methods used, it should be emphasized that Kaplan-Meier analysis has its limitations. Some limitations can be overcome, such as censoring, which is limited in our study by having a follow-up of ≥ 5 years. In this study, the terminal event was defined as death by any cause, which may result in a lower survival rate than Mountain[2] reported because he defined the terminal event as "death from cancer or unknown cause." Moreover, our definition was chosen because of the retrospective character of the analysis, which included data from a 23-year period, during which the cause of death was not always well documented, and because of possible insufficient diagnosis in any end-stage disease.

As stated previously, the best survival is found in patients with T1N0M0 and the worst survival in T3N2M0. Other TNM subsets have intermediate survival rates, and staging is still debated.[2,8,10,24] Because the staging system is designed to guide the choice of a therapeutic regimen, the staging system was built on clinical data. However, staging with the use of exclusive clinical data is not always exact, as chest physicians do experience in daily practice. Clinical staging turns out to be understaging in a considerable number of patients when surgical data become available for these patients. This can also be derived from the clinical and surgical data of Mountain, in which understaging is most notable in stages IB and IIA. Although we agree that the staging system is meant to guide therapy and to give prognosis and is initially based on clinical data, we want to discuss the staging system after analysis of postsurgical data. In stage II, the subset pT1N1M0 has a better survival than the other subsets, supporting the division of stage II into stages IIA and IIB. However, no significant difference in survival is found between stage IB and stage IIA. This latter observation was also made by Inoue et al[8] as well as by Rami-Porta.[10] The similar survival rates for stages IB and IIA can be found in both the clinical and the postsurgical data of Mountain[2]; because surgery is advocated for both stage IB and stage IIA, use of two separate substages can be disputed.

Despite inclusion of T3N0M0 in stage IIB, which is supported by survival rates similar to T2N1M0, stage IIIA still represents an inhomogeneous group with regard to both anatomical extent of tumor and survival. T3 has a rather broad definition, including patients with tumor extension in very different tissues, allowing different possibilities for (curative) surgery.[5,9,13,24] For this reason, inclusion of T3N1M0 is also under discussion—even more so, because in our study, survival was found not to be significantly different from T3N0M0 survival. By latter observation, downstaging of T3N1M0 to stage IIB is suggested. However, patients with T3 tumors invading the thoracic wall have better survival than T3 patients with central localization.[24] The present T definition does not allow separation of these groups of patients with thoracic wall involvement vs central localization.[24] Apart from T3 tumors, stage IIIA also includes N2 disease, for which treatment with adjuvant chemotherapy is now advocated. For clinical and therapeutic reasons, inclusion of N2 disease in one stage (IIIA) is supported.

In conclusion, the TNM staging system accurately reflects the prognosis in primary NSCLC, but some stage definitions can be discussed. Despite the fact that the staging system is built on clinical data to assess treatment in individual patients, the present analysis, which includes postsurgical data, confirms the similar survival of patients with T2N0M0 and T1N1M0. This finding stresses the importance of using two separate substages, especially because these patients are offered surgery when possible.

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Development of protegrins for the treatment and prevention of oral mucositis: structure-activity relationships of synthetic protegrin analogues.

Chen J, Falla TJ, Liu H, Hurst MA, Fujii CA, Mosca DA, Embree JR, Loury DJ, Radel PA, Cheng Chang C, Gu L, Fiddes JC.

IntraBiotics Pharmaceuticals, Inc., 1255 Terra Bella Avenue, Mountain View, CA 94043, USA.

Protegrin antimicrobial peptides possess activity against gram-positive and gram-negative bacteria and yeasts. An extensive structure-activity relationship (SAR) study was conducted on several hundred protegrin analogues to gain understanding of the relationship between the primary and secondary structure of the protegrins and their antimicrobial activities, and to identify a protegrin analogue for clinical development. Native sequence protegrins are cationic, amphiphilic peptides that are characterized by the presence of a beta-sheet structure that is maintained by two disulfide bridges. The presence of the beta-sheet is key to the stability of the protegrin structure; linearized analogues or analogues that have amino acid substitutions that eliminate hydrogen bonding across the beta-sheet have reduced activity, especially in the presence of physiological concentrations of NaCl. Also, maintaining amphiphilicity of the beta-sheet is key; analogues with substitutions of polar amino acids in the hydrophobic face have reduced activity. Analogues with reduced positive charge tend to be less active, an observation that is more marked for gram-negative than gram-positive bacteria, and may implicate binding to lipopolysaccharide as a key mechanistic step in the killing of gram-negative bacteria. A very large number of amino acid substitutions are tolerated by the protegrin structure, implying that overall structural features such as amphiphilicity, charge, and shape are more important to activity than the presence of specific amino acids. This lack of importance of specific stereochemistry is supported by the fact that completely D-amino acid substituted protegrins are fully potent. Based on the SAR studies, and on the microbiological data from an animal model, one protegrin analogue, IB-367, was selected for clinical development as a

topical agent to prevent the oral mucositis associated with cancer therapy.

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SMI's 2nd Annual Superbugs and Superdrugs Conference: innovations in anti-infectives.

Barrett JF.

Bristol-Myers Squibb, Wallingford, CT 06492, USA.
John.Barrett@bms.com

The emergence of the 'Superbugs', resistant bacterial pathogens, is being aggressively met by the anti-infective community, both academia and industry, with an assortment of classical and novel approaches to control these resistant pathogens. The launch of improved quinolones (gatifloxacin and moxifloxacin), the launch of a new class of protein synthesis inhibitors (oxazolidinones; linezolid) and the ushering-in of the applied genomics age, all offer hope for the future control of resistant bacteria. The seemingly imminent development and completion of the first lipopeptide, daptomycin, offers great hope for the control of Gram-positive resistant pathogens. The first cationic peptide, IB-367, designed to combat the niche medical need of mucositis and the development of a specific antistaphylococcal glycopeptide. BI-397, all will precede the first wave of genomic-targets-based drug candidates, as the antimicrobial genomics effort remains in the target identification and validation stages of early discovery.

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IB-367, a protegrin peptide with in vitro and in vivo activities against the microflora associated with oral mucositis.

Mosca DA, Hurst MA, So W, Viajar BS, Fujii CA, Falla TJ.

IntraBiotics Pharmaceuticals, Inc., Mountain View, California 94043, USA.

Although the microflora associated with oral mucositis initiated by cytotoxic therapy is not well characterized, several studies suggest that reduction of the microbial load in the oral cavity has some clinical benefit. The MICs of IB-367, a synthetic protegrin analog, ranged from 0.13 to 64 microgram/ml for gram-positive bacteria (Streptococcus mitis, Streptococcus sanguis, Streptococcus salivarius, and Staphylococcus aureus) and from 0.06 to 8 microgram/ml for gram-negative species (Klebsiella, Escherichia, and Pseudomonas). IB-367 exhibited rapid, microbicidal activity against both log- and stationary-phase cultures of methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa. At concentrations near the MICs for these two organisms (4 and 2 microgram/ml, respectively), IB-367 reduced viability by more than 3 logs in less than 16 min. Similarly, IB-367 effected a 4-log reduction of the endogenous microflora in pooled human saliva within 2 min at 250 microgram/ml, a concentration readily attained by local delivery. After nine serial transfers at 0.5x the MIC, the MIC of IB-367 for MRSA and P. aeruginosa increased only two to four times. In a phase I clinical study with healthy volunteers, IB-367 was well tolerated, with no detectable systemic absorption. One hour after treatment with 9 mg of IB-367, the prevalence of gram-negative bacteria and yeast was reduced, and the density of the predominant gram-positive oral flora was decreased 1,000 times. IB-367's properties (speed of killing, breadth of spectrum, and lack of resistance) make the compound a strong candidate for the prophylaxis of oral mucositis. Phase II clinical trials with IB-367 are under way for this indication in immunocompromised subjects.

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IB-367, a Protegrin Peptide with In Vitro and In Vivo Activities against the Microflora Associated with Oral Mucositis

DEBORAH A. MOSCA,† MALINDA A. HURST, WENDY SO, BEVERLY S. C. VIAJAR, CRAIG A. FUJII, AND TIMOTHY J. FALLA*

IntraBiotics Pharmaceuticals, Inc., Mountain View, California 94043

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Although the microflora associated with oral mucositis initiated by cytotoxic therapy is not well characterized, several studies suggest that reduction of the microbial load in the oral cavity has some clinical benefit. The MICs of IB-367, a synthetic protegrin analog, ranged from 0.13 to 64 μg/ml for gram-positive bacteria (*Streptococcus mitis*, *Streptococcus sanguis*, *Streptococcus salivarius*, and *Staphylococcus aureus*) and from 0.06 to 8 μg/ml for gram-negative species (*Klebsiella*, *Escherichia*, and *Pseudomonas*). IB-367 exhibited rapid, microbicidal activity against both log- and stationary-phase cultures of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. At concentrations near the MICs for these two organisms (4 and 2 μg/ml, respectively), IB-367 reduced viability by more than 3 logs in less than 16 min. Similarly, IB-367 effected a 4-log reduction of the endogenous microflora in pooled human saliva within 2 min at 250 μg/ml, a concentration readily attained by local delivery. After nine serial transfers at 0.5× the MIC, the MIC of IB-367 for MRSA and *P. aeruginosa* increased only two to four times. In a phase I clinical study with healthy volunteers, IB-367 was well tolerated, with no detectable systemic absorption. One hour after treatment with 9 mg of IB-367, the prevalence of gram-negative bacteria and yeast was reduced, and the density of the predominant gram-positive oral flora was decreased 1,000 times. IB-367's properties (speed of killing, breadth of spectrum, and lack of resistance) make the compound a strong candidate for the prophylaxis of oral mucositis. Phase II clinical trials with IB-367 are under way for this indication in immunocompromised subjects.

The microflora of the mouth has a complex and diverse ecology. Although more than 200 species of microorganisms have been isolated from the oropharynx, individual surfaces of the oral cavity are dominated by specific subgroups (12). For example, alpha and nonhemolytic viridans group streptococci predominate on the surface of the buccal mucosa (13). The development of illnesses such as cancer has been associated with significant shifts in the numbers of gram-negative bacteria detectable in oral samples (18, 21). An increase in the levels of *Candida albicans* can also occur; this species is the cause of most oral fungal infections in cancer patients.

The cytotoxic effects of radiation therapy or chemotherapy on rapidly dividing epithelial cells of the oropharyngeal mucosa often lead to a very painful condition known as oral mucositis (5, 26). Lesions associated with oral mucositis are usually found on the buccal and sublingual mucosae (17). Infections with endogenous microflora or opportunistic pathogens are thought to exacerbate this condition, leading to ulceration, inflammation, and accumulation of microorganisms in the necrotic tissue (3, 4, 11, 25). Although the microflora associated with oral mucositis is not well characterized, a reduction in the microbial load of the oral cavity appears to have some benefit in the treatment of oral mucositis in cancer patients (2, 6, 22, 24). Currently, no effective U.S. Food and Drug Administration-approved therapy exists for prevention or treatment of oral mucositis (14, 18).

We have tested the antimicrobial activity of a synthetic protegrin analog, IB-367 ($RGGLCYCRGRFCVCVGR_{CONH2}$), against representatives of the most prevalent groups of aerobic oral flora using a modified version of the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (15, 23). We have also determined the MICs for multiple strains of aerobic gram-negative species most commonly associated with oral mucositis (*Klebsiella*, *Serratia*, *Escherichia*, and *Pseudomonas*) and gram-positive species associated with accompanying complications such as bacteremia (*Streptococcus mitis* and *Streptococcus sanguis*) or systemic shock (*Staphylococcus aureus*). In addition, the ability of IB-367 to reduce the level of the oral microflora in vivo was determined in a phase I clinical trial performed with healthy volunteers.

We demonstrate here that IB-367 exhibits properties that may be vital for effective treatment of oral mucositis: (i) broad-spectrum antimicrobial activity, (ii) rapid killing, and (iii) a relative lack of resistance development.

MATERIALS AND METHODS

Bacterial strains. Strains of gram-positive bacteria obtained from the American Type Culture Collection (ATCC; Rockville, Md.) included methicillin-sensitive *S. aureus* (MSSA; Smith type; ATCC 19636 and ATCC 29213), methicillin-resistant *S. aureus* (MRSA; ATCC 33591), vancomycin-sensitive *Enterococcus faecalis* (ATCC 29212) and *Enterococcus faecium* (ATCC 19434), *Streptococcus salivarius* (ATCC 7073, ATCC 31067), *Streptococcus mitis* (ATCC 9811, ATCC 15914), and *Streptococcus pneumoniae* (ATCC 49619). Gram-negative bacteria included *Acinetobacter calcoaceticus* (ATCC 17905, ATCC 23055), *Escherichia coli* (ATCC 25922, ATCC 23579), *Haemophilus influenzae* (ATCC 49247), *Klebsiella pneumoniae* (ATCC 10031, ATCC 9997), *Neisseria meningitidis* (ATCC 13093), *Pseudomonas aeruginosa* (ATCC 9027, ATCC 27853, ATCC 39324), and *Serratia marcescens* (ATCC 13880). Two strains of *C. albicans* (ATCC 10231 and ATCC 90029) were also tested. Additional clinical isolates were obtained from Patricia Mickelson, Clinical Microbiology Laboratory, Stanford University, Calif. API test strips (BioMerieux, Hazelwood, Mo.) were used to confirm organism identities, and strains were stored frozen in 10% glycerol at −80°C.

Media for in vitro assays. Mueller-Hinton broth (MHB), cation-adjusted Mueller-Hinton broth II, Trypticase soy broth (TSB), and Trypticase soy agar

* Corresponding author. IntraBiotics Pharmaceuticals, Inc., 1255 Terra Bella Ave., Mountain View, CA 94043. Phone: (650) 526-6800. Fax: (650) 969-0663. E-mail: tfalla@intrabiotics.com.

† Present address: Elitra Pharmaceuticals, San Diego, CA 92121.

(TSA) were purchased in powder form from Becton Dickinson, Cockeysville, Md., and were prepared in distilled, deionized water. Haemophilus test medium (HTM) and RPMI medium without $NaHCO_2$ but with 20 mM HEPES and L-glutamine (0.3 g/liter) were purchased premade from Becton Dickinson and Sigma Chemical Co. (St. Louis, Mo.), respectively. Blood agar plates (BAPs) containing TSA with 5% sheep blood added, MacConkey agar plates, Sabouraud dextrose agar plates, mannitol salt agar plates, and horse blood were purchased from Hardy Diagnostics, Santa Maria, Calif. Lysed horse blood (LHB) was prepared by mixing a thawed sampled of frozen blood 1:1 with sterile water, centrifuging, and adding the supernatant to MHB at 2% as needed for adequate growth. Liquid Testing Medium (LTM) contained the following: 10 mM phosphate buffer (pH 6.5), 1% TSB, and 100 mM NaCl. Phosphate-buffered saline (PBS) contained 10 mM phosphate (pH 7.4) and 100 mM NaCl.

Reagents. Norfloxacin (95% pure by high-performance liquid chromatography [HPLC]), vancomycin (1,118 μg/mg), polymyxin B (7,760 U/mg), and gentamicin (647 μg/mg) were obtained from Sigma Chemical Co., and ciprofloxacin (867 μg/mg) was obtained from Bayer Corporation (Kankakee, Ill.). Human saliva was obtained from healthy volunteers after brushing. IB-367 was synthesized in-house with Rink-amide resins, by 9-fluorenylmethoxy carbonyl solid-phase chemistry, and with an automated peptide synthesizer (model 431A; Applied Biosystems, Foster City, Calif.). The peptide was cleaved from the solid support with trifluoroacetic acid and was subsequently folded by using air oxidation. The peptide was purified by reverse-phase HPLC (Vydac C_{18} column; 2.2 by 25 cm; solvent A, 0.1% trifluoroacetic acid in water; solvent B, 0.08% trifluoroacetic acid in acetonitrile; linear gradient, 21 to 49% solvent B in 30 min; flow rate, 8 ml/min; detection at 214 nm).

In vitro susceptibility testing. MICs were determined by a slightly modified version of the NCCLS broth microdilution method as described previously (23). Briefly, antimicrobial agents were prepared as 10× concentrates in the most appropriate solvent. For IB-367, 0.01% acetic acid containing 0.2% bovine serum albumin as a carrier protein was used. Vancomycin, polymyxin B, ciprofloxacin, and gentamicin were dissolved in water, whereas norfloxacin was dissolved in 100% dimethyl sulfoxide and was then serially diluted in water. Inocula were prepared by resuspending colonies from a BAP in medium and adjusting the suspension to match that of a 0.5 McFarland standard. The suspension was diluted into fresh medium (as recommended by NCCLS for the organism) to give 2×10^5 to 7×10^5 CFU/ml for bacteria or 2×10^3 to 7×10^3 CFU/ml for *Candida*. After dispensing 100-μl aliquots of the microbial suspension into each well of a 96-well polypropylene microtiter plate, 11 μl of test compound was added. The MIC was defined as the lowest concentration of drug which prevented visible turbidity after 16 to 20 h (bacteria) or 46 to 50 h (*Candida*) at 35°C. Minimum bactericidal concentrations (MBCs) or minimum fungicidal concentrations were determined by transferring 10 μl from each clear well (greater than or equal to the MIC) onto a BAP. After incubation for 20 h, the MBC was identified as the lowest concentration that did not permit any visible growth on the surface of the agar.

Resistance studies. MRSA and *P. aeruginosa* were harvested from the well with an IB-367 concentration equal to 0.5× the MIC, diluted to 1×10^5 to 5×10^5 CFU/ml in fresh MHB, and dispensed into microtiter plates as 100-μl aliquots. Compounds were added as described above, and MICs were determined daily for up to 18 serial passages. MICs were also determined for cultures incubated 5 days before serial passage.

Microbicidal assays. Bacteria were grown overnight in TSB (10 ml in a 50-ml Erlenmeyer flask) at 200 rpm and 37°C to the stationary phase. Stationary-phase cultures in TSB were centrifuged, resuspended in PBS (MRSA) or LTM (*P. aeruginosa*) at 4×10^5 CFU/ml, and then dispensed into polypropylene microcentrifuge tubes. Exponential-phase cultures were prepared by diluting an overnight culture in MHB into fresh MHB (1:1,000) and incubating the culture at 200 rpm and 37°C until an absorbance (at 600 nm) of 0.2 was reached. The culture was then diluted to 1×10^5 to 4×10^5 CFU/ml in prewarmed MHB, reincubated to allow two cell doublings, and then dispensed into polypropylene microcentrifuge tubes. After addition of the test compounds at a 1/10 volume, the tubes were incubated without aeration at the appropriate temperature. Fresh human saliva was mixed 1:1 with peptide (10 mM sodium acetate buffer [pH 5]) or conventional antimicrobial agents (sterile water), and the mixture was then incubated at 37°C without aeration. The numbers of viable CFU were determined by one of two methods. By the pour plate method, 20-μl aliquots of serial dilutions in 0.87% saline were mixed with approximately 20 ml of tempered (50°C) TSA. By the spread plate method, 20-μl aliquots of serial dilutions in 0.87% saline were spread onto the surface of the desired agar plate. Both methods allowed rapid sampling, minimized drug carryover (i.e., ≤0.01× the MIC), and precluded the need for washing of the cells to remove the drug. The plates were incubated for 18 to 24 h at the appropriate temperature, and the colonies were enumerated to determine the microbicidal effect of the drug.

Phase I clinical trial. The study population consisted of healthy men and women (ages, 18 to 65 years) who were within 20% of their ideal weight range for age, height, and frame and who were nonsmokers. Use of antibiotics within 28 days of study entry was not allowed, nor was the use of over-the-counter medications and commercially available mouthwashes for at least 14 days prior to the start of the study. In addition, subjects had to be willing and able to refrain from consuming alcohol- or caffeine-containing foods and beverages during the study.

TABLE 1. Comparison of in vitro activity of IB-367 with those of other antimicrobial agents

Compound	MIC (μg/ml)	
	MRSA (ATCC 33591)	*P. aeruginosa* (ATCC 9027)
IB-367	4	2
Norfloxacin	0.5	0.25
Gentamicin	1.0	0.5
Vancomycin	1.0	NA[a]
Polymyxin B	NA	0.5

[a] NA, not applicable.

Subjects were excluded from the study if they had a history of anaphylaxis or xerostomia or if lesions of the oral mucous membranes were present.

The phase I trial was a multiple-ascending-dose study. The subjects received 3 g of IB-367 gel formulation four times daily for 4 consecutive days. Groups of six subjects each received a gel formulation containing either 0.3, 1, or 3 mg of IB-367 per g of gel. The subjects were asked to hold the gel in their mouth without spitting or swallowing for 5 min. In addition, they were asked not to eat or drink for 1 h after receiving the study medication. Samples of oral microflora were taken prior to administration of the first dose and 1 h after administration of the first dose on days 1, 2, and 4 of treatment. Samples were obtained and the microbial content was determined by a modified version of an oral washing method described previously (19). Briefly, the oral cavity was washed with 20 ml of sterile saline from which 10-fold serial dilutions were made in sterile saline containing 0.1% Tween 80. The total number of aerobic bacteria present was determined by plating each dilution onto BAP. The presence of gram-negative bacteria, staphylococcal species, and yeast was determined by plating 100 μl of the undiluted sample onto MacConkey agar, mannitol salt agar, and Sabouraud agar plates, respectively. All agar plates were incubated overnight at 35°C, and the numbers of viable organisms were determined as the numbers of CFU per milliliter.

RESULTS

In vitro susceptibility. The MICs of IB-367 and conventional antimicrobial agents for MRSA and *P. aeruginosa* in MHB were determined (Table 1). When a strain of MRSA was tested in MHB, growth was acceptable and the MIC of IB-367 was 4 μg/ml. When the same strain was tested in MHB plus 2% LHB or HTM, the MIC of IB-367 increased to 16 μg/ml.

Clinical isolates representing aerobic organisms prevalent in the oral cavity were tested for their susceptibilities to IB-367 (Table 2). The MICs of IB-367 ranged from 0.13 to 64 μg/ml for gram-positive bacteria and from 0.06 to 8 μg/ml for gram-negative bacteria. One exception was *S. marcescens*, for which MICs were in the range of 16 to 256 μg/ml. In general, the MBCs of IB-367 for all organisms were within 1 dilution of the MICs (data not shown).

Bactericidal activity. The bactericidal activity of IB-367 was compared to those of conventional antimicrobial agents at concentrations near their respective MICs. After the addition of IB-367 to stationary-phase cultures of MRSA (Fig. 1A) or *P. aeruginosa* (Fig. 1B), the numbers of viable CFU of both organisms decreased by 3 log units within 8 min. Polymyxin B produced a similar, rapid reduction in the numbers of *P. aeruginosa* CFU, whereas vancomycin was not bactericidal against MRSA in this time period. Gentamicin and norfloxacin required ≥2 h to effect the same decrease in the numbers of *P. aeruginosa* CFU and were completely ineffective against MRSA at this concentration.

When tested against log-phase cultures of MRSA growing in MHB, IB-367 reduced the numbers of CFU by 4 log units within 4 min, whereas gentamicin, norfloxacin, and vancomycin were completely ineffective, even after 2 h (Fig. 2A). Similar results were observed for exponentially growing cultures of *P. aeruginosa* (Fig. 2B). IB-367 reduced the numbers of CFU

TABLE 2. Broad-spectrum antimicrobial activity of IB-367 against oral flora by the modified NCCLS broth microdilution method[a]

Group and organism (no. of strains)	Prevalence range (%)[b]	MIC range (μg/ml)
Gram-positive bacteria		
Viridans group streptococci[c]	93–99	
S. salivarius (12)	50–75	0.2–5.0
S. sanguis (14)	25–75	4–64
S. mitis (15)	25–75	2–43
S. mutans (3)	25–75	0.7–1.3
Streptococcus spp., group D (6)	90–100	0.25–4
Streptococcus spp. (16)[c]	1–50	1.3–16
Corynebacterium spp. (5)[c]	15–90	0.13–0.25
Propionibacterium	11–12	NT[d]
Staphylococcus spp. (35)	3–70	0.13–4
Lactobacillus	1–37	NT
Gram-negative bacteria		
Moraxella spp. (12)	81–97	0.2–0.8
Neisseria sp. (1)[c]	5–97	8
Haemophilus spp. (15)[c]	5–35	1–8
Acinetobacter calcoaceticus (4)	5–30	0.06–2
Klebsiella pneumoniae (4)	5	1–5
Pseudomonas aeruginosa (18)	5	1–8
Escherichia coli (5)	NA[e]	0.25–1
Serratia marcescens (16)	NA	16–>256
Yeast, *Candida albicans* (6)	3–6	4–16

[a] Described in reference 15.
[b] Adapted from Liljemark and Bloomquist (12).
[c] Addition of blood products (e.g., 2% LHB or hematin) is required for acceptable growth.
[d] NT, not tested.
[e] NA, not applicable.

more rapidly than either norfloxacin or gentamicin did, and the reduction achieved with IB-367 was similar to the reduction achieved with polymyxin B.

Although increasing the concentrations of conventional antimicrobial agents to 16 μg/ml improved the reduction in the numbers of CFU in both log- and stationary-phase cultures of *P. aeruginosa*, the reductions did not equal that achieved with IB-367 (data not shown). In contrast, the higher concentration did not affect the reduction in the numbers of CFU when the conventional antimicrobial agents were tested against MRSA.

Microbicidal activity against heterogeneous flora in saliva. IB-367 exhibited concentration-dependent microbicidal activity against the oral microflora when it was mixed 1:1 with pooled human saliva from healthy volunteers (Fig. 3). At 250 μg/ml, IB-367 effected a 4-log reduction in the levels of the endogenous oral microflora within 2 min, whereas vehicle alone did not exhibit any antimicrobial effect. When compared with conventional antimicrobial agents at 1,000 μg/ml, IB-367 reduced the numbers of CFU by >4 log units in 1 min (Fig. 4). In contrast, tobramycin and ciprofloxacin reduced the numbers of CFU by 1 log unit after 16 and 60 min, respectively, whereas vancomycin was essentially ineffective even after 2 h.

Evaluation of resistance. When cultures of MRSA or *P. aeruginosa* were serially transferred daily, the MICs of norfloxacin increased as much as 32 times, whereas little change was observed in the MICs of vancomycin, polymyxin B, or IB-367 (data not shown). In a second study, the incubation period before subculture was extended from 18 to 20 h to 5 days to enhance the detection of low-frequency mutations that might engender resistance. Under these conditions, the initial MICs of norfloxacin for *P. aeruginosa* and MRSA were slightly higher (1 and 2 μg/ml, respectively) compared with the MICs determined after the standard incubation of 18 to 20 h (0.25 and 0.5 μg/ml, respectively). After nine serial transfers, the MICs of norfloxacin increased 160 times for *P. aeruginosa* and 320 times for MRSA (Fig. 5). In contrast, the MIC of IB-367 for each strain increased only four times. As before, the MICs of polymyxin B and vancomycin were relatively unaffected.

Efficacy of IB-367 in reducing oral microflora. IB-367 reduced the density of the oral microflora in human volunteers in a concentration-dependent manner by as much as 1,000 times (Fig. 6). The 3-mg/g dose reduced the level of gram-negative bacteria, staphylococcal species, and yeast to undetectable (<10 CFU/ml) levels in all subjects (Table 3). No serious adverse effects, no evidence of allergic or anaphylactoid reactions, and no clinically significant changes in vital signs were observed during the study. In addition, IB-367 remained effective against mixed oral microflora after repeated exposures (Fig. 6).

DISCUSSION

In this study, we describe the in vitro and in vivo properties of IB-367, a synthetic protegrin analog that prompted its development as a preventive treatment for oral mucositis. The

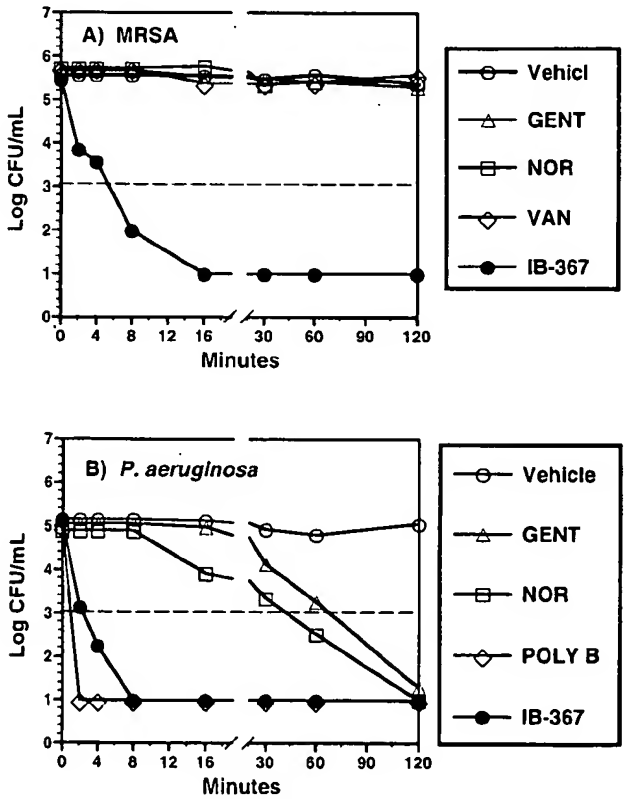

FIG. 1. Bactericidal activity of IB-367 against stationary-phase cultures. After overnight growth in TSB, stationary-phase cultures of MRSA or *P. aeruginosa* were resuspended in either PBS (pH 7.4) (A) or LTM (B). Except for IB-367 (4 μg/ml), all compounds were added at 1 μg/ml, and survivors were enumerated at the indicated times by the pour plate method. The dashed line represents the minimum number of CFU per milliliter which could be accurately determined. GENT, gentamicin; NOR, norfloxacin; VAN, vancomycin; POLY B, polymyxin B; Vehicle, buffer or medium plus the test organism.

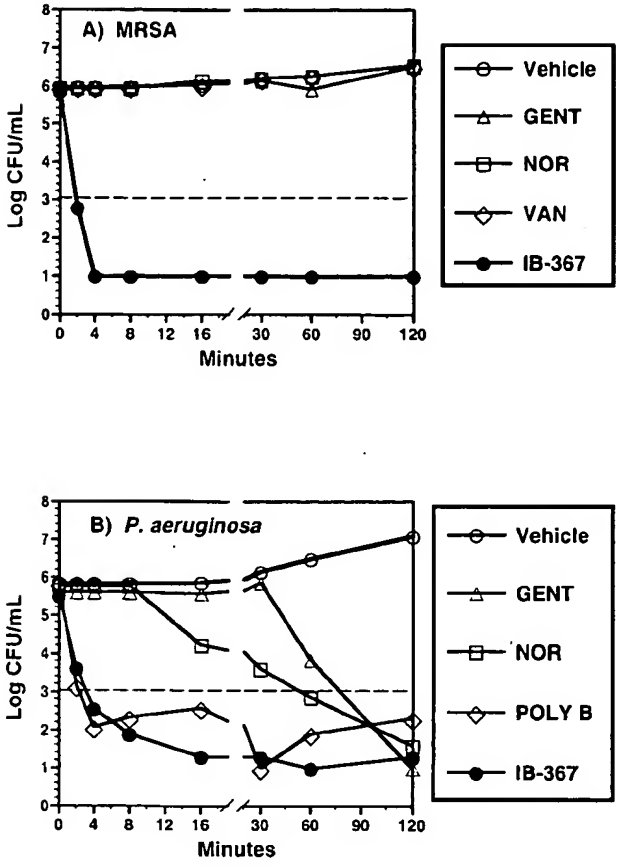

FIG. 2. Bactericidal activity of IB-367 against log-phase cultures. Exponentially growing MRSA (A) or *P. aeruginosa* (B) was treated with IB-367 (4 μg/ml) or other antimicrobial agents at 1 μg/ml. Survivors were enumerated at the indicated times by the pour plate method. The dashed line represents the minimum number of CFU per milliliter which could be accurately determined. GENT, gentamicin; NOR, norfloxacin; VAN, vancomycin; POLY B, polymyxin B; Vehicle, buffer or medium plus test organism.

MICs of IB-367 for MRSA (ATCC 33591) and *P. aeruginosa* (ATCC 9027) were slightly higher than those of conventional antimicrobial agents. However, IB-367 exhibited good to excellent antimicrobial activity against many of the microorganisms associated with the oral cavity, including *C. albicans*. In some cases, the elevated MICs may actually represent interference from the addition of blood products to the growth medium. Although such supplements are required for adequate growth of several fastidious organisms such as *S. mitis* and *S. sanguis* (2 to 43 and 4 to 64 μg/ml, respectively), they affect the biological activity of IB-367. Since the MICs increased approximately four times in MHB containing LHB or HTM, one might consider that the true MIC of IB-367 for organisms that require these medium supplements may be as much as four times lower than those reported in Table 1.

Extension of the incubation period from 18 to 24 h to 5 days prior to subculture of MRSA or *P. aeruginosa* produced a substantial increase in the MICs of norfloxacin. However, only a small increase in the MICs of IB-367 was observed. Such a low level of resistance is unlikely to be of clinical significance as the increases in the MICs were negligible compared to the concentration of IB-367 administered to the oral cavity in clinical trials. These data demonstrate that the development of significant resistance of bacteria to IB-367 is highly unlikely,

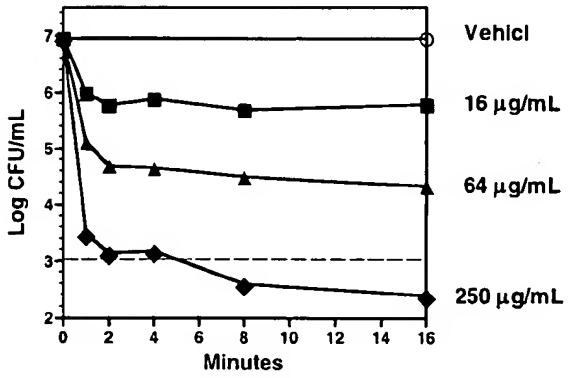

FIG. 3. Microbicidal activity of IB-367 against polymicrobial flora in saliva from healthy human volunteers. IB-367 (10 mM sodium acetate [pH 5]) was mixed 1:1 with saliva to give the indicated final concentrations. Vehicle contained 10 mM sodium acetate (pH 5) mixed 1:1 with saliva. At the indicated intervals, aliquots were spread onto the surfaces of TSA plates containing 10% fetal bovine serum, and the plates were incubated overnight at 37°C for 48 h prior to enumeration of the number of survivors. The dashed line represents the minimum number of CFU per milliliter which could be accurately determined.

regardless of the incubation conditions. In contrast, resistance to conventional antibiotics such as the fluoroquinolones develops quite easily. Overall, these data imply that the rapid generation of high-level resistance to IB-367 in clinical use is unlikely.

The properties of an agent required for successful treatment of oral mucositis include a broad spectrum of activity, activity that is not compromised by saliva and that can be achieved despite a short contact time with the pathogen, and demonstrated product safety. As described here, IB-367 conforms to all of these criteria. In addition, we have demonstrated that IB-367 can significantly reduce the number of bacteria and yeast that colonize the oral mucosa, a phenomenon previously demonstrated to reduce the severity of oral mucositis and its sequelae (24).

Various antimicrobial agents have been evaluated for their potential to reduce the level of oral mucositis. The narrow-

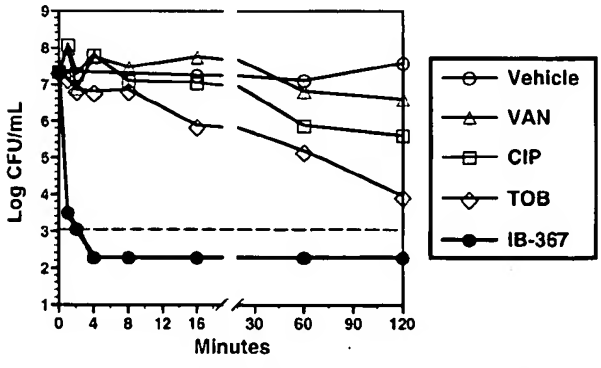

FIG. 4. Comparison of microbicidal activity of IB-367 and conventional antimicrobial agents against polymicrobial flora in saliva from healthy human volunteers. Saliva was mixed 1:1 with test compound (IB-367; 10 mM sodium acetate [pH 5]; vancomycin [VAN], ciprofloxacin [CIP], or tobramycin [TOB] in sterile deionized water) to a final concentration of 1,000 μg/ml. Vehicle contained 10 mM sodium acetate (pH 5) mixed 1:1 with saliva. At the indicated intervals, aliquots were plated onto TSA containing 5% sheep's blood, and the plates were incubated overnight at 37°C for 24 h prior to enumeration of the number of survivors. The dashed line represents the minimum number of CFU per milliliter which could be accurately determined.

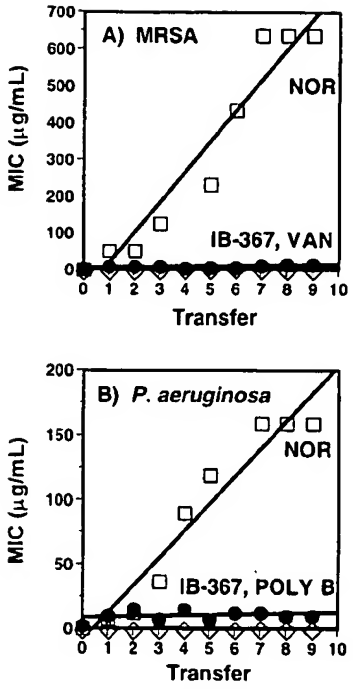

FIG. 5. Effect of serial transfer on MICs. Cultures of MRSA (A) or *P. aeruginosa* (B) were exposed to various concentrations of drugs. After 5 days of incubation, wells containing compounds at concentrations equal to one-half the MIC were subcultured into fresh medium containing the same drugs. NOR, norfloxacin; VAN, vancomycin; POLY B, polymyxin B.

spectrum antimicrobial agents evaluated in clinical studies include clindamycin (6) and nystatin (7), both of which failed to change the local course or modify the systemic sequelae of oral mucositis. Chlorhexidine, a broad-spectrum microbicide, was initially shown to reduce the severity of mucositis as a complication of bone marrow transplantation in a single-center study (8). The efficacy of chlorhexidine was not, however, confirmed in a subsequent study in patients undergoing bone marrow transplant (7, 27) or in patients experiencing oral mucositis as a complication of radiation therapy (20). One possible explanation for these negative results is inactivation of chlorhexidine

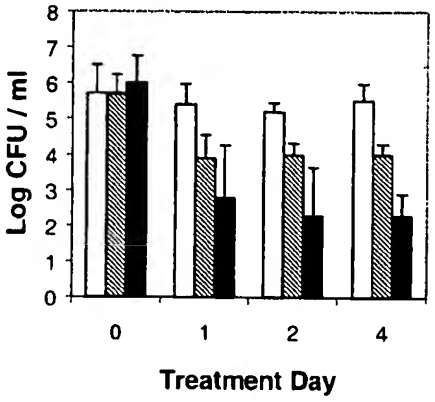

FIG. 6. Reduction of oral microflora in human volunteers by IB-367. IB-367 was administered at three doses: 0.3 mg/g (unfilled bars), 1.0 mg/g (shaded bars), and 3.0 mg/g (filled bars). The buccal mucosa was sampled on day 1 prior to treatment (time 0) and 1 h after treatment on days 1, 2, and 4.

TABLE 3. Prevalence of oral microflora before treatment and 1 h after treatment with IB-367

Dose (mg/g)	Prevalence (%)					
	Gram-negative bacteria		Staphylococcal species		Yeast	
	Before	After	Before	After	Before	After
0.3	33	0	66	0	67	33
1.0	33	0	50	0	50	33
3.0	16	0	100	0	84	0

by saliva, resulting in a failure to achieve a reduction in the microbial burden in the mouth (20).

Higher concentrations of IB-367 were required for effective reduction of the heterogeneous oral flora in pooled normal human saliva. The presence of many negatively charged glycoproteins such as mucin in saliva may bind to the positively charged peptide, rendering it less bioavailable (1, 16). In addition, the increased microbial density in saliva (ca. 5×10^7 CFU/ml) has a substantial inoculum effect on the MIC. However, the levels of IB-367 required for effective reduction of the numbers of CFU (250 to 1,000 μg/ml) are readily achieved in topical formulations of the peptide, as demonstrated by the significant reduction in the oral microflora of human volunteers.

In a multicenter study of patients receiving radiation therapy, administration of chlorhexidine was found to increase oral discomfort compared to administration of a placebo (10). That study was stopped prematurely after interim analysis demonstrated increased oral toxicity. In contrast, the current phase I study of IB-367 demonstrated that the 3-mg/g dose was well tolerated and showed no serious adverse effects. It should also be noted that plasma IB-367 concentrations were below the limit of detection by HPLC-mass spectrometric analysis (<16 ng/ml), indicating little or no systemic absorption of the compound (data not shown).

Conventional antibiotics such as tobramycin or vancomycin have also been used in topical applications for the treatment of oral mucositis with limited success (2, 22). Even at concentrations 1,000 times higher than their MICs, tobramycin, vancomycin, and ciprofloxacin were incapable of exhibiting the rapid reduction in the numbers of CFU in saliva demonstrated by IB-367. These data suggest that IB-367 may offer substantial advantages over conventional antimicrobial agents that require bacterial growth or a longer period of exposure.

IB-367 is a potent, broad-spectrum, rapidly microbicidal agent with superior performance in saliva compared with those of conventional antimicrobial agents and is capable of reducing the prevalence of the oral microflora in humans. IB-367 is currently in phase II clinical trials for the prophylaxis of oral mucositis in bone marrow transplant patients undergoing ablative chemotherapy.

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     FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT
     16:20:33 ON 06 DEC 2003
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L1              0 S E3 AND REBECCA
L2              0 S E3 AND FUCHS
L3              0 S E3 AND VAP
L4              0 S E3 AND IB-367
L5           3423 S  VAP
L6              2 S L5 AND IB-367

     FILE 'STNGUIDE' ENTERED AT 16:24:03 ON 06 DEC 2003
L7              0 S IB-367

     FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT
     16:26:07 ON 06 DEC 2003
L8            108 S IB-367
L9             17 S L8 AND RESPIRATORY
L10            13 DUP REM L9 (4 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 16:27:53 ON 06 DEC 2003

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E2            1     REDMAINDER/BI
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E9            1     REDMANS/BI
E10           1     REDMAPLE/BI
E11           8     REDMAR/BI
E12           1     REDMARKING/BI

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L3            0 REDMAN/BI AND VAP

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L4            0 REDMAN/BI AND IB-367

=> s  VAP
L5         3423 VAP

=> s l5 and IB-367
L6            2 L5 AND IB-367

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L6 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:509435 BIOSIS
DOCUMENT NUMBER: PREV200200509435
TITLE: A Phase IIa safety and microbial kinetic study of iseganan (**IB-367**) oral solution in intubated patients receiving mechanical ventilation.
AUTHOR(S): Kollef, M. [Reprint author]; Redman, R.; Jensen, K.; Mertens, R. [Reprint author]
CORPORATE SOURCE: Washington Univ. School of Medicine, Saint Louis, MO, USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2001) Vol. 41, pp. 403. print. Meeting Info.: 41st Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Illinois, USA. September 22-25, 2001.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Slide)
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Oct 2002
Last Updated on STN: 5 Dec 2002

AB Background: Iseganan, previously known as **IB-367**, is a synthetic analog of the protegrin antimicrobial peptides with broad activity against gram-positive and gram-negative bacteria and yeast and low potential for resistance. We conducted a Phase IIa, multi-center, placebo (pbo) controlled study evaluating the potential of iseganan oral solution (soln) as an oral decontaminant for prevention of ventilator associated pneumonia (**VAP**). Methods: Sixteen orally intubated patients (pts) were randomized to receive 9 mg iseganan (n=6) or pbo soln (n=2) every (q) 4 hours (h), or 9 mg iseganan (n=6) or pbo soln (n=2) q 6 h for up to 5 days. For each administration, 3 mL of study drug were applied to all surfaces of the oral cavity and retained for at least 5 minutes. Oral secretions were collected pre- and post-dose for quantitative microbial analysis q 24 h. Results: Iseganan oral soln was well tolerated. Immediate decreases in mean oral microbial burden were seen after administration of the 1st dose of each day: overall mean decrease for each day pts were intubated were 1.0 log colony forming units (CFU) for iseganan and 0.1 log CFU for pbo (p=0.01). A cumulative decrease was seen over 5 days of administration for iseganan q 4 h (n=3; 3.2 log CFU) vs pbo (n=2; 0.9 log CFU). Data where oral CFUs were below the limit of detection and thus suspect to technical error were excluded from statistical analyses. Conclusions: Oral-topical administration of iseganan soln safely and rapidly reduces the oral microbial burden of orally intubated and mechanically ventilated pts. Decreases in oral microbial burden are observed daily with both q 4 h and q 6 h dosing; cumulative decreases over 5 days of treatment are seen with q 4 h administration. Iseganan soln is a promising broad spectrum single agent candidate for the prevention of **VAP**.

L6 ANSWER 2 OF 2 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2002-674861 [72] WPIDS
DOC. NO. CPI: C2002-190066
TITLE: Prevention of respiratory infections associated with intubation or mechanical ventilation by administration of a composition comprising **IB-367** peptide.
DERWENT CLASS: A96 B04
INVENTOR(S): FUCHS, H; REDMAN, R
PATENT ASSIGNEE(S): (FUCH-I) FUCHS H; (REDM-I) REDMAN R; (INTR-N) INTRABIOTICS PHARM INC
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002064089	A2	20020822	(200272)*	EN	22

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW

US 2003073625 A1 20030417 (200329)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002064089	A2	WO 2002-US4362	20020212
US 2003073625	A1 Provisional	US 2001-268585P	20010213
		US 2002-75924	20020212

PRIORITY APPLN. INFO: US 2001-268585P 20010213; US 2002-75924 20020212

AN 2002-674861 [72] WPIDS

AB WO 200264089 A UPAB: 20021108

NOVELTY - Prevention of a respiratory infection associated with intubation or mechanical ventilation involves topically applying a composition comprising an **IB-367** peptide or its salt to the oral cavity of the patient.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for preventing ventilator-associated pneumonia (**VAP**) involving applying an aqueous composition comprising (wt.%) native **IB-367** peptide or its salt (preferably hydrochloride) (0.3), sorbitol (10), xylitol (3), hydroxypropyl methylcellulose (0.2), lactic acid (0.1), methyl paraben (0.18), propyl paraben (0.02) and water (balance) to the oral cavity of the patient. The composition has a pH of about 4.

ACTIVITY - Antimicrobial; Respiratory-Gen; Antiinflammatory.

MECHANISM OF ACTION - None given.

USE - For preventing a respiratory infection associated with intubation or mechanical ventilation (preferably ventilator-associated pneumonia (**VAP**)) (claimed).

ADVANTAGE - The method can be applied without worrying about creating resistant strains of pathogens. The method decreases the time spent on the ventilator. The composition has ability to rapidly and broadly lower the bacterial load in the oral and oropharyngeal cavities.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	33.91	34.12

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FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Nov 28, 2003 (20031128/UP).

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L7          0 IB-367
              (IB(W)367)

=> FIL BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                0.18      34.30

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=> s IB-367
L8          108 IB-367

=> s l8 and respiratory
L9           17 L8 AND RESPIRATORY

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10          13 DUP REM L9 (4 DUPLICATES REMOVED)

=> d l10 ibib abs 1-13

L10  ANSWER 1 OF 13  EMBASE  COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
ACCESSION NUMBER:    2003469119  EMBASE
TITLE:               Therapeutic peptides.
AUTHOR:              Lien S.; Lowman H.B.
CORPORATE SOURCE:    H.B. Lowman, Dept. of Protein Engineering, Genentech, 1 DNA
                     Way, South San Francisco, CA 94080, United States.
                     hbl@gene.com
SOURCE:              Trends in Biotechnology, (2003) 21/12 (556-562).
                     Refs: 82
                     ISSN: 0167-7799  CODEN: TRBIDM
COUNTRY:             United Kingdom
DOCUMENT TYPE:       Journal; General Review
FILE SEGMENT:        008     Neurology and Neurosurgery
                     016     Cancer
                     030     Pharmacology
                     037     Drug Literature Index
                     039     Pharmacy
LANGUAGE:            English
SUMMARY LANGUAGE:    English
AB   Novel peptide therapeutics are increasingly making their way into clinical
     application. Indeed, certain naturally derived peptides have been
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successful drugs for many years. With the advent of large biological and synthetic peptide libraries and high-throughput screening, many promising candidates could soon be added to the list of peptides under development. These advances have introduced new strategies for the administration of peptide drugs and improvements of clearance half-lives in vivo. Despite the potential obstacles that remain, peptide therapeutics are poised to play a significant role in the treatment of diseases ranging from Alzheimer's disease to cancer.

L10 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:637478 CAPLUS
DOCUMENT NUMBER: 137:179854
TITLE: Methods of preventing ventilator associated pneumonia by oral administration of antimicrobial **IB-367** peptides
INVENTOR(S): Redman, Rebecca; Fuchs, Henry
PATENT ASSIGNEE(S): Intrabiotics Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 22 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002064089	A2	20020822	WO 2002-US4362	20020212
WO 2002064089	A3	20021212		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003073625	A1	20030417	US 2002-75924	20020212

PRIORITY APPLN. INFO.: US 2001-268585P P 20010213

AB The present invention provides methods of preventing **respiratory** infections assocd. with intubation and/or mech. ventilation-assocd. pneumonia, in intubated patients. The method generally involves topical administration of a compn. comprising an **IB-367** peptide to the oral cavity of an intubated patient. As **IB-367** peptides engender very little resistance, a significant advantage of the invention is that the prophylactic therapy may be applied without having to worry about creating resistant strains of pathogens. A single dose of native IB-36 oral rinse (compn. described) was active against Gram-pos. and Gram-neg. bacteria and yeasts in orally intubated patients, and showed no serious adverse events.

L10 ANSWER 3 OF 13 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003087901 EMBASE
TITLE: **Respiratory** drug development compendium 2002.
AUTHOR: Graul A.I.
SOURCE: Drugs of the Future, (1 Dec 2002) 27/12 (1181-1194).
ISSN: 0377-8282 CODEN: DRFUD4
COUNTRY: Spain
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
037 Drug Literature Index

LANGUAGE: English

L10 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2002:621913 CAPLUS
DOCUMENT NUMBER: 137:179266
TITLE: Iseganan (IntraBiotics Pharmaceuticals)
AUTHOR(S): Toney, Jeffrey H.
CORPORATE SOURCE: Merck Research Laboratories, Rahway, NJ, 07065-0900, USA
SOURCE: Current Opinion in Investigational Drugs (PharmaPress Ltd.) (2002), 3(2), 225-228
CODEN: COIDAZ; ISSN: 1472-4472
PUBLISHER: PharmaPress Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review. Iseganan (**IB-367**) is a protegrin under development by IntraBiotics, as part of a larger protegrin program, for the potential treatment of oral mucositis, a frequent side effect of anticancer therapies. The company is developing three formulations of the drug: A rinse for the potential treatment of mucositis, an aerosolized liq. for the potential treatment of **respiratory** infection and a gel formulation for the potential treatment of pneumonia [376325]. Iseganan kills a broad-spectrum of bacteria and fungi, including those resistant to conventional antimicrobial drugs, by attaching to and destroying the integrity of the lipid cell membrane [241594]. Until August 1999, Pharmacia & Upjohn was a codeveloper of iseganan. IntraBiotics re-acquired the global rights to iseganan in Dec. 1999, and both companies agreed to terminate the collaboration [335766]. In May 2000, analysts at SG Cowen predicted the drug's potential market at US $100 to US $200 million [376325].
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 13 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003356390 EMBASE
TITLE: Iseganan: **IB 367**, Protegrin **IB 367**.
SOURCE: Drugs in R and D, (2002) 3/1 (52-55).
Refs: 6
ISSN: 1174-5886 CODEN: DRDDFD
COUNTRY: New Zealand
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
011 Otorhinolaryngology
037 Drug Literature Index
038 Adverse Reactions Titles
039 Pharmacy
LANGUAGE: English

L10 ANSWER 6 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:509435 BIOSIS
DOCUMENT NUMBER: PREV200200509435
TITLE: A Phase IIa safety and microbial kinetic study of iseganan (**IB-367**) oral solution in intubated patients receiving mechanical ventilation.
AUTHOR(S): Kollef, M. [Reprint author]; Redman, R.; Jensen, K.; Mertens, R. [Reprint author]
CORPORATE SOURCE: Washington Univ. School of Medicine, Saint Louis, MO, USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2001) Vol. 41, pp. 403. print.

Meeting Info.: 41st Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Illinois, USA. September 22-25, 2001.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Slide)
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Oct 2002
Last Updated on STN: 5 Dec 2002

AB Background: Iseganan, previously known as **IB-367**, is a synthetic analog of the protegrin antimicrobial peptides with broad activity against gram-positive and gram-negative bacteria and yeast and low potential for resistance. We conducted a Phase IIa, multi-center, placebo (pbo) controlled study evaluating the potential of iseganan oral solution (soln) as an oral decontaminant for prevention of ventilator associated pneumonia (VAP). Methods: Sixteen orally intubated patients (pts) were randomized to receive 9 mg iseganan (n=6) or pbo soln (n=2) every (q) 4 hours (h), or 9 mg iseganan (n=6) or pbo soln (n=2) q 6 h for up to 5 days. For each administration, 3 mL of study drug were applied to all surfaces of the oral cavity and retained for at least 5 minutes. Oral secretions were collected pre- and post-dose for quantitative microbial analysis q 24 h. Results: Iseganan oral soln was well tolerated. Immediate decreases in mean oral microbial burden were seen after administration of the 1st dose of each day: overall mean decrease for each day pts were intubated were 1.0 log colony forming units (CFU) for iseganan and 0.1 log CFU for pbo (p=0.01). A cumulative decrease was seen over 5 days of administration for iseganan q 4 h (n=3; 3.2 log CFU) vs pbo (n=2; 0.9 log CFU). Data where oral CFUs were below the limit of detection and thus suspect to technical error were excluded from statistical analyses. Conclusions: Oral-topical administration of iseganan soln safely and rapidly reduces the oral microbial burden of orally intubated and mechanically ventilated pts. Decreases in oral microbial burden are observed daily with both q 4 h and q 6 h dosing; cumulative decreases over 5 days of treatment are seen with q 4 h administration. Iseganan soln is a promising broad spectrum single agent candidate for the prevention of VAP.

L10 ANSWER 7 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:555326 BIOSIS
DOCUMENT NUMBER: PREV200200555326
TITLE: In vitro activity of iseganan (**IB-367**) against bacterial isolates obtained from patients with cystic fibrosis (CF) enrolled in a phase I clinical trial.
AUTHOR(S): Fujii, C. [Reprint author]; Boggs, A. [Reprint author]; Stapp, J.; Burns, J. L.; Redman, R. [Reprint author]
CORPORATE SOURCE: IntraBiotics Pharmaceuticals, Inc, Mountain View, CA, USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2001) Vol. 41, pp. 200. print.
Meeting Info.: 41st Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Illinois, USA. September 22-25, 2001.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Oct 2002
Last Updated on STN: 30 Dec 2002

AB Background: Iseganan, previously known as **IB-367**, is a synthetic analog of the protegrin antimicrobial peptides. In 1999/2000, a multi-center, multi-dose, placebo-controlled clinical trial was conducted to study the safety and potential utility of aerosolized delivery of iseganan in controlling the bacterial burden in the lungs of adult patients with CF. Methods: More than 250 bacterial isolates obtained from

patients enrolled in this study were analyzed at the CF lab at CHRMC for susceptibility to iseganan, ceftazidime (CAZ), ciprofloxacin (CIP), colistin (COL), tobramycin (TOB), vancomycin (VAN), andoxacillin (OXA). MICs were determined by broth microdilution per NCCLS; for iseganan, the compound was diluted in 0.01% acetic acid in the presence of BSA, but other parameters were per NCCLS. Results: For iseganan, the MIC90 against P. aeruginosa isolates was 8 mug/mL (n=222) and the MIC90 against S. aureus was 4 mug/mL (n=48). Iseganan MICs ranged from 2-16 mg/mL vs. S. maltophilia (n=9), 1-16 mug/mL vs. A. xylosoxidans (n=4), and 2->64 mug/mL vs. B. cepacia (n=2) MIC ranges for other drugs vs. P. aeruginosa (n=222) were as follows: CAZ, 0.25->64 mg/mL; CIP, COL, and TOB, 0.06->64 mug/mL. MICs vs. S. aureus (n=47) ranged from 0.25-1 mg/mL for VAN and 0.12-64 mug/mL for OXA. Cross-resistance of iseganan to the other drugs tested was not evident. Iseganan MICs were similar for isolates retrieved from patients prior to therapy versus isolates retrieved either after receiving iseganan therapy or placebo. Conclusions: Iseganan shows good in vitro activity against clinical isolates of pathogens relevant to the CF community, does not present immediate resistance or cross-resistance, and remains an interesting candidate for further development.

L10 ANSWER 8 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:555231 BIOSIS
DOCUMENT NUMBER: PREV200200555231
TITLE: Bactericidal mechanism of iseganan (**IB-367**), a rapidly acting antimicrobial protegrin peptide.
AUTHOR(S): Orlov, D. [Reprint author]; Wang, V. W. [Reprint author]; Hong, T. [Reprint author]; Menzel, L. P. [Reprint author]; Azimov, R. [Reprint author]; Falla, T. J.; Waring, A. J. [Reprint author]; Lehrer, R. I. [Reprint author]
CORPORATE SOURCE: UCLA School of Medicine, Los Angeles, CA, USA
SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2001) Vol. 41, pp. 99. print. Meeting Info.: 41st Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Illinois, USA. September 22-25, 2001.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Oct 2002
Last Updated on STN: 30 Dec 2002

AB Background: **IB-367**, a rapidly acting antimicrobial peptide, is currently in clinical trials for prevention of oral mucositis and ventilator-associated pneumonia. Methods: To ascertain the mechanism of action, we made quantitative measurements on living bacteria, did transmission and scanning electron microscopy, and studied the peptide's effects in model liposome and planar bilayer systems. Results: **IB-367** bound to LPS and caused simultaneous permeabilization of E. coli's outer and inner membranes, allowing transit of molecules up to 600-750 daltons. This process began with a massive, unregulated influx of water, and a compensatory release of K+-rich intracellular fluid. Continued operation of osmotic forces caused bacterial swelling to begin within a few minutes, increasing the effective diameter of protegrin-induced membrane channels, and causing portions of the inner membrane to extrude through clefts (tesserae) in the peptidoglycan and form protrusions, approximately 70-100 ANG in diameter. The protrusions extended beyond the peptidoglycan and were osmotically fragile unless the medium contained impermeant osmolytes (e.g, polyethylene glycols) whose hydrodynamic radii exceeded 10ANG. Without such osmotic stabilizers, the surface protrusions of E. coli ruptured explosively, releasing cytoplasmic contents and leaving annular scars on the bacterial surface. Similar extrusions and cytoplasmic ruptures were observed when staphylococci were

treated, although more often the cytoplasmic ruptures were contained by their thicker cell walls. Conclusions: Iseganan (**IB-367**) uses a novel mechanism: "HOTTER" (Hydro-Osmotic Trans-Tesseral Extrusion and Rupture), to deliver a dramatic and irreversible coup de grace to Gram-positive and Gram-negative bacteria.

L10 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:176198 BIOSIS
DOCUMENT NUMBER: PREV200200176198
TITLE: Identification and characterization of Burkholderia cepacia mutants sensitive to antimicrobial peptides.
AUTHOR(S): Gunn, J. S. [Reprint author]; McCoy, A. J. [Reprint author]; Tran, L. T. [Reprint author]; Liu, H.; Falla, T.
CORPORATE SOURCE: Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 22. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Mar 2002
Last Updated on STN: 6 Mar 2002

AB Burkholderia cepacia, a common pathogen of the lungs of cystic fibrosis patients, is highly resistant to the action of a number of antimicrobial peptides (AP). AP are important components of the innate defenses of animals, plants, and microorganisms. A transposon mutagenesis approach was undertaken to identify B. cepacia genes involved in AP resistance. Approximately 17,000 mutants were screened for increased susceptibility to polymyxin B (PM), resulting in the identification of four PM-sensitive mutants. Minimal inhibitory concentration assays demonstrated these mutants to range from 2- to 64-fold more sensitive that the parental strain to PM. Two of the mutants also showed decreased resistance to the protegrin analog, **IB-367** (4- and 16-fold increased sensitivity). DNA sequence analysis of the mutagenized loci revealed similarities to an unknown protein whose gene is flanked by putative lipopolysaccharide (LPS) biosynthetic genes, TolB, and AcfD, a putative stearol-acyl-carrier-protein desaturase thought to be involved in the production of a B. cepacia lipopeptide antibiotic. Silver stained LPS profiles of the mutants sensitive to PM and **IB-367**, but not the mutants sensitive to PM only, show marked migration differences in the lipid A/core region. The exact nature of these lipid A/core alterations were determined by mass spectrometry and paper chromatography. These data further support the role of LPS as the major factor in the resistance of gram-negative bacteria to the action of antimicrobial peptides.

L10 ANSWER 10 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:176150 BIOSIS
DOCUMENT NUMBER: PREV200200176150
TITLE: Pseudomonas aeruginosa outer membrane proteins and **IB-367**.
AUTHOR(S): Jain, P. [Reprint author]; Liu, H. [Reprint author]; Fujii, C. A. [Reprint author]; Tran, L. [Reprint author]; Boggs, A. F. [Reprint author]; Falla, T. J. [Reprint author]
CORPORATE SOURCE: Intrabiotics Pharmaceuticals, Inc., Mountain View, CA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 11. print.
Meeting Info.: 101st General Meeting of the American

Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Mar 2002
Last Updated on STN: 6 Mar 2002

AB **IB-367** (RGGLCYCRGRFCVCVGRCONH2) is an antimicrobial peptide currently under development for the prophylaxis of oral mucositis and the treatment of **respiratory** infections. We have previously demonstrated that Pseudomonas aeruginosa OprL, a membrane stabilizing protein, is over-expressed in response to a single challenge with **IB-367**. To determine if such adaptation is responsible for reducing the susceptibility of P.aeruginosa to **IB-367** over time, we analyzed strains following extensive serial passage in the presence of the peptide. P.aeruginosa was passaged in 0.5X the MIC of **IB-367** every 24 hours until any increase in MIC had stabilized for at least 10 days. Following 40 days of passaging, total cellular protein from two strains, exhibiting a 4 fold increase in MIC, were subjected to 2D-SDS-PAGE analysis to identify any significant changes in protein expression. Proteins were identified by mass spectral analysis of their trypsin digests compared to those of known proteins. No change in the cellular content of OprL was observed in 2D gel analysis. However, the cellular levels of another outer membrane protein, OprG, were significantly reduced in both serial passage strains relative to the parent. No other significant changes were observed. In this study, we have demonstrated that although OprL appears up-regulated in response to a single challenge with **IB-367**, a reduction in the OprG content of the outer membrane is responsible for a decrease in susceptibility of P.aeruginosa. A reduction in the OprG content of the P.aeruginosa outer membrane has previously been shown to be associated with reduced susceptibility to a wide range of antibiotics (quinolones, beta-lactams, tetracyclines and chloramphenicol). This is the first report of this non-specific ability of P. aeruginosa with respect to a cationic peptide.

L10 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2000:190910 CAPLUS
DOCUMENT NUMBER: 132:231949
TITLE: Combined therapy for treatment of inflammation using elastase inhibitor(s) and antibacterial agent(s)
INVENTOR(S): Maiti, Samarendra N.; Phillips, O. A.; Salama, Sameeh; Micetich, Ronald G.
PATENT ASSIGNEE(S): Naeja Pharmaceutical Inc., Can.
SOURCE: PCT Int. Appl., 15 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000015207	A2	20000323	WO 1999-IB1547	19990915
WO 2000015207	A3	20000525		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9955282 A1 20000403 AU 1999-55282 19990915

PRIORITY APPLN. INFO.: US 1998-100386P P 19980915
WO 1999-IB1547 W 19990915

AB A method of treatment is provided to treat inflammation assocd. with human neutrophil elastase-mediated disorders, particularly diseases of **respiratory** system or oral cavity. The method comprises a combined therapy of administering an elastase inhibitor(s) with an antibacterial agent(s), directed against pathogenic bacteria assocd. with disease of the **respiratory** system and oral cavity. A pharmaceutical compn. comprising at least one antibacterial agent, and at least one elastase inhibitor is provided, preferably in aerosolized form, as well as a kit contg. the resp. components in sep. packages.

L10 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:206857 BIOSIS
DOCUMENT NUMBER: PREV200000206857
TITLE: Prognostic assessment of 2,361 patients who underwent pulmonary resection for non-small cell lung cancer, stage I, II, and IIIA.
AUTHOR(S): van Rens, Marcel Th. M.; Brutel de la Riviere, Aart; Elbers, Hans R. J.; van den Bosch, Jules M. M. [Reprint author]
CORPORATE SOURCE: Department of Pulmonary Diseases, Sint Antonius Hospital, 3430 EM, Nieuwegein, Netherlands
SOURCE: Chest, (Feb., 2000) Vol. 117, No. 2, pp. 374-379. print. CODEN: CHETBF. ISSN: 0012-3692.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 May 2000
Last Updated on STN: 5 Jan 2002

AB Study objectives: Staging and classification in lung cancer are important for both patient management and clinical research. Results of survival after resection in patients with primary non-small cell lung cancer (NSCLC) are analyzed in order to validate recent refinements of the staging system. Design: Retrospective study; period from 1970 to 1992; follow-up gtoreq 5 years. Patients: A total of 2,361 previously untreated patients who underwent resection for stage I, II, or IIIA primary NSCLC. Measurements: Survival was estimated from the date of operation using the Kaplan-Meier survival analysis method. Deaths within 30 days of operation were excluded. Survival comparisons of different surgical-pathologic TNM classification (based on pathologic examination of resected specimens) as well as further discriminative factors were analyzed by log-rank test. Results: Postoperative death occurred in 3.9% of patients. For survival analyses, 2,263 patients were included. The overall 5-year survival was 937/2,263 (41.4%). Five-year survival in stage IA was 255/404 (63%); in stage **IB, 367**/797 (46%); in stage IIA, 43/83 (52%); in stage IIB, 210/642 (33%); and in stage IIIA, 63/337 (19%). No significant difference in survival was demonstrated between stages IB and IIA. Until 4 years after surgery, age at operation did not influence survival; after 5 years, patients > 65 years old had a significantly lower survival. Conclusion: The TNM staging system accurately reflects the prognosis in primary NSCLC, but some stage definitions can be discussed. Despite the fact that the staging system is built on clinical data, the present analysis, which includes postsurgical data, confirms the similar survival of patients with T2N0M0 and T1N1M0. These results also stress the use of two separate substages, especially because these patients are offered surgery when possible.

L10 ANSWER 13 OF 13 EMBASE

on STN
ACCESSION NUMBER: 2000138901 EMBASE
TITLE: Cationic antimicrobial peptide antibiotics.
AUTHOR: Hancock R.E.W.; Nair P.
CORPORATE SOURCE: R.E.W. Hancock, Dept. of Microbiology and Immunology, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. bob@cmdr.ubc.ca
SOURCE: Current Opinion in Anti-infective Investigational Drugs, (2000) 2/2 (140-144).
Refs: 39
ISSN: 1464-8458 CODEN: COADFY
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 037 Drug Literature Index
030 Pharmacology
004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Cationic antimicrobial peptides are found throughout nature and are important components of the innate defenses of host organisms against infectious agents. They have very desirable properties as antimicrobials, with some peptides having broad spectrum activity against both Gram-negative and Gram-positive bacteria, fungi and enveloped viruses, as well as anti-endotoxic activity and synergy with conventional antibiotics. Although these peptides are quite large, generally 13 to 26 amino acids in length, recombinant synthesis methods are being created to moderate the cost of manufacture. Variants of natural peptides are currently being developed as therapeutics against topical infections, and two peptides have successfully proceeded through phase II clinical trials.

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(FILE 'HOME' ENTERED AT 16:20:22 ON 06 DEC 2003)

FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT 16:20:33 ON 06 DEC 2003
E REDMAN
L1 0 S E3 AND REBECCA
L2 0 S E3 AND FUCHS
L3 0 S E3 AND VAP
L4 0 S E3 AND IB-367
L5 3423 S VAP
L6 2 S L5 AND IB-367

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     FILE 'STNGUIDE' ENTERED AT 16:24:03 ON 06 DEC 2003
L7             0 S IB-367

     FILE 'BIOSIS, CAPLUS, MEDLINE, WPIDS, EMBASE, SCISEARCH' ENTERED AT
     16:26:07 ON 06 DEC 2003
L8           108 S IB-367
L9            17 S L8 AND RESPIRATORY
L10           13 DUP REM L9 (4 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 16:27:53 ON 06 DEC 2003

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